April 20, 1971

Dr. M. G. P. Stoker
Imperial Cancer Research Fund
Lincoln's Inn Fields
London, W.C. 2

Dear Michael,

I ordinarily don't respond so quickly, particularly when the distractions are as many as they are now. Nevertheless, I did want to say a bit about the conA binding studies.

You may recall when we talked in Paris or in London, I can't remember which, I told you of our inability to confirm a difference in the binding of conA to normal and transformed cells. We never liked Sachs' experiments with the nickel labeled conA' because we were worried about whether he was measuring nickel binding rather than conA binding to cells (the radioactive nickel was not covalently bound to the conA and, in fact, is readily dissociable by dialysis). His data could easily have been explained if transformed cells were a more efficient ion exchanger than normal cells. We used ^{125}I-labeled conA prepared enzymatically and could detect no difference in binding even though the labeled conA preparation caused agglutination of transformed cells but not of normal cells. I told this to Joe Sambrook in Paris, but he had repeated Sachs' experiment with nickel labeled conA and obtained results similar to those of Sachs. Subsequently, he also prepared ^{125}I-labeled conAAand wheat germ agglutinin and obtained the same results as we have. At first I was somewhat reluctant to believe Donna Arndt's data because it was at variance with our expectations; nevertheless, she has tried in a variety of ways to find a difference but without success; and Joe's confirmation of her findings makes me convinced that we're not wrong and that there has to be another explanation for the differential agglutinability.

It's not a technical problem, we're not using either glass filters or Millipore filters. Our first set of experiments were done with cell suspensions made with EDTA which were mixed with labeled conA, incubated under various conditions, centrifuged, washed several times, and then the cells were dissolved and counted. Burger raised the objection that perhaps our EDTA treatment was liberating some protease perhaps from 1% of the cells and this obliterated the difference. We accepted his suggestion for doing

the experiment in the following way: monolayers are washed and then exposed to varying concentrations of labeled conA directly on the plate. After an appropriate time the layer is washed several times and then dissolved in dilute alkali and aliquots of the alkaline solution are counted and the protein content determined. Again, there is no appreciable difference based on protein content or cell number in conA binding.

Joe wrote me recently saying that some people in your lab have found a similar result and I assume that's what you refer to in your letter.

I can imagine several ways to reconcile our results with the differential agglutinability. It seems to me one could imagine that there are steric barriers in normal cells which prevent agglutination even though conA is bound; perhaps a divalent conA molecule cannot cross link normal cells but can do so with transformed or trypsinized cells. Conceivably, the cell surface of transformed cells undergoes an allosteric change following conA binding and this allosteric change makes the cells "sticky". If normal cells don't undergo the allosteric change on conA binding, they might not become "sticky". A third possibility is that the charge on the surface of normal cells prevents the agglutination even though conA is bound. If the charge on transformed cells is altered (and there is evidence that this is so), there might be no charge repulsion to prevent agglutination. I am sure there are other possible explanations, but at the moment I am inclined to believe that there is no such thing as cryptic conA binding sites in normal cells.

All the best,

Sincerely,

PS.: If we can make it to the Cancer Gordon Conference, we'll look forward to seeing you.